Aflatoxin Producing Moulds And Aflatoxin Residues In Meat and Meat Products In Egypt

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ABSTRACT

This investigation was designed to throw light on the contamination rate of meat and meat products with mould and aflatoxins. The prevalence and population density of the mycobiota of 350 beef meat and meat product samples (frozen meat, minced meat, liver, kidney, luncheon, sausage, hawawchi), 50 of each, collected from different abattoirs, markets and shopkeepers in Egypt were studied. The highest total mould count/g was obtained from the sausage samples ($4.20 \pm 1.25 \times 10^4$), whereas the frozen meat samples yielded the lowest mould count ($2.00 \pm 1.2 \times 10^2$). The most frequently encountered mould genera from the examined samples were Aspergillus and penicillium. *Aspergillus* species were isolated at percent of 100%, 80%, 80%, 78%, 74%, 70% and 50% from minced meat, hawawchi, susage, luncheon, liver, frozen meat and kidney samples respectively. *Aspergillus flavus* was the most predominant isolated species. All *A. parasiticus* and 77.96% of *A. flavus* isolates were positive for aflatoxin production on coconut agar medium (CAM), thin layer chromatography (TLC) and fluorometric immune affinity method. Detection of aflatoxin residues in meat and meat products samples revealed that the highest mean values of aflatoxin residues (µg/kg) B_1, B_2, G_1 and G_2 were detected in the kidney samples (12.36 ± 1.89, 9.84 ± 1.63, 5.38 ± 1.36 and 6.84 ± 1.39, respectively)followed by the liver (13.81 ± 1.96, 3.26 ± 0.92, 2.51 ± 0.63 and 1.36 ± 0.38), luncheon (3.71 ± 1.35, 3.59 ± 1.12, 5.24 ± 1.12 and 6.77 ± 1.49), hawawchi (11.03 ± 2.43, 2.25 ± 0.52, 2.54 ± 0.99 and 2.56 ± 0.27), minced meat (3.62 ± 0.88, 3.40 ± 0.82, 4.24 ± 0.85, 2.83 ± 0.60) and frozen meat samples which had the lowest level of AFs (4.80 ± 0.89, 5.3 ± 2.1, 1.71 ± 0.60 and 0.0) respectively. The detected levels of aflatoxin residues in the present samples were compared with the international permissible limits of WHO, FAO and FDA. Most of detected aflatoxins levels in meat and meat product samples were more than the permissible limits thus would be unfit for human consumption. The present study attracts the attention to potential risk for aflatoxin producing moulds and aflatoxins contamination and strongly recommends reduction in various causes of contamination as lack of hygienic measures during slaughtering, handling, transportation, storage or processing to meat and its products and also avoiding contaminated spices used in meat processing.

Key words: Aflatoxin producing moulds, aflatoxins, thin layer chromatography, fluorometric immune affinity method.

INTRODUCTION

Disease outbreaks due to the consumption of contaminated food and feedstuff are a recurring problem worldwide. The major factor contributing to contamination are microorganisms, especially fungi, which produce low-molecular-weight compounds as secondary metabolites, with confirmed toxic properties referred to as mycotoxins. Several mycotoxins reported to date are cosmopolitan in distribution and incur severe health-associated risks (including cancer and neurological disorders) *(1)*.Aflatoxins are the most important mycotoxins, produced by *Aspergillus flavus* and *Aspergillus parasiticus* strains and pose a quadruple threat to both

human and animals as they produce four distinct effects: acute liver damage, liver cirrhosis, induction of tumors and teratogenic effects *(2, 3)*. Residues of these toxins in animal tissues and their products are a public health concern. There are a number of moulds that have been described as pathogens. These are particularly found in meat products that have ripened for a long time, such as sausages and hams. *Penicillium* species, *Aspergillus* species, and *Cladosporium* species have been identified in such products *(4)*.

The conditions of the environment in the manufacturing rooms, stores, refrigerators and shops are very suitable for the development of moulds inside the products, but more frequently on the surface of various sorts of meat and meat products *(5)*. When the temperature and relative humidity are optimal after mould contamination, there is also a risk of mycotoxin production *(6)*. Relatively low water activity (aw< 0.9) and low pH-values (pH < 6.0) are particularly favorable for mould development *(7)*. However, the environmental conditions for mycotoxin production are generally more restricted than those for mould growth *(8)*. In addition, the toxigenic potential of moulds is directly dependent on the substrate on which they are grown, and mycotoxin synthesis is lower in meat substrates than in media with a higher amount of carbohydrates *(9-11)*. This highlights that the growth of the toxigenic moulds on the surface of hams does not always indicate the presence of corresponding mycotoxin *(12)*. However, AFB1, B2 and G1 have been reported in fresh and processed meat products in Egypt as a consequence of the growth of *A. flavus* and *A. parasiticus* strains *(13)*.It is worth to mention that heat treatment as cooking and roasting aflatoxins in food unable to destroy *(14)*.Hence, creating awareness among consumers, as well as developing new methods for mycotoxin detection and inactivation is of great importance for food safety *(1)*. In this study, the focus is on the occurrence of various types of moulds with special reference to aflatoxigenic species, their screening for aflatoxin producing ability and aflatoxin residues in meat and meat products associated with risks to humans.

MATERIAL AND METHODS

Samples

Three hundred and fifty samples (350) belonging to 6 kinds of beef meat and meat products (frozen meat, minced meat, liver, kidney, luncheon, sausage, hawawchi), 50 of each, were collected from different abattoirs, markets and shopkeepers in Egypt. Samples were aseptically transferred into sterile polyethylene bags undue delay and transported to the laboratory for mycological examination and aflatoxins detection.

Mycological analysis

Enumeration of total mould count

Preparation of samples

Ten grams of each sample were transferred aseptically into sterile blender jar, to which 90 ml of 1% peptone water were added and homogenized in a sterile warring blender for 2 minutes, and ten fold serial dilutions of the homogenate were prepared *(15)*. One millimeter quantities of the previously prepared serial dilutions were inoculated separately into Petri dish plates and mixed with Saboraud dextrose agar medium. The plated were the left to solidify after mixing, and incubated at 25°C for 3-5 days. The counts of mould colonies were recorded. Individual suspected colonies were selected depending upon their morphological characters. Stock culture were made from each isolate and monitored on Czapeks Dox, malt extract and potato dextrose (PDA) agar slopes for further identification.

Identification of isolates

The identification of mould species were carried out by observation of macroscopic and microscopic characteristics of the mould colonies according to *(16)* for genus Aspergillus and and other genera were identified according to *(17)*.

Aflatoxin analysis

Fluorescence screening on coconut agar media (CAM)

*A. flavus*and *A. parasiticus* strains were cultured on CAM at 25°C for 7 days in the

dark. Cultures were observed under long-wave UV light (365 nm) after 3, 5 and 7 days and the presence or absence of fluorescence on the reverse side of the colonies was recordedas reported ***(18)***.

Extraction of aflatoxin

Extraction of aflatoxin from *A. flavus* and *A. parasiticus* isolates according to ***(19, 20)***.

*A. flavus*and *A. parasiticus* strains were subcultured on PDA slants for approximately 7 days at (25°C) until well sporulated. Spores were harvested by adding 5 ml sterile, double distilled water and dislodging the spores aseptically with a sterile inoculating loop. The final spore concentration was adjusted to be approximately 1.5 x 10^8 spore/ml. Spore suspension (500ul) was inoculated into each flask having 25 ml of sterile yeast extract-sucrose (2% yeast extract, 15% sucrose) and supplemented with 0.019 % *P. cresol.* Inoculated flasks were incubated at 25°C in the dark for 20 days. Mycelia were overlaid with 25 ml chloroform and kept 24 hours in dark. Then 25 ml chloroform was added again and cultures were shaken for ½ an hour. Chloroform layers were filtered into 500 ml round bottom flasks and cultures were extracted once more with 50 ml chloroform. Chloroform layers were combined and concentrated in a rotatory flash evaporator.

Extraction of aflatoxins from meat and its products samples ***(21)***.

Each sample was finally ground in an electric mill to pass sieve No. 10.

Twenty five grams of the ground sample were transferred into a 500 ml Erlenmeyer flask, then covered with a thin layer of solid 6 mm diameter glass beads and extracted with a solution composed of 90 ml acetonitrile and 10 ml of 4% potassium chloride solution. The mixture was shaken vigorously for 30 minutes and filtered through 9 cm Whatman No. 41 which allows rapid filtration with minimum solvent evaporation. 50 ml of filtrate was defattened by shaking with an equal volume of iso-octane (2, 2, 4-trimethyl pentone) in 250 ml separating funnel. When the two layers were clearly separated, the upper layer was discarded and the lower layer re-extracted with another 50 ml iso-octane followed by discarding the lipid extract. Distilled water (12.5 ml) was added to the acetonitrile layer and aflatoxin B1 was extracted with 25 ml chloroform. The lower acetonitrile-chloroform layer was drained through a bed of anhydrous sodium sulphate contained in funnel lined with Whatman No. 41 paper. Extraction of aqueous layer was repeated 3 more times using 10 ml portions of chloroform. Filtrates were combined and evaporated to dryness in a rotatory evaporator.

Qualitative estimation of aflatoxins by thin layer chromatography (T.L.C.) according to ***(22)*** and inspected under U.V. light (256 nm and 365 nm) and the outline of each fluorescence spot was marked by a sharp pin. The Rf values, colours and intensities of the unknown spots were compared with those of standard spot.

Quantitative estimation of aflatoxins by a fluorometeric immunoaffinity method ***(22)***.

Statistical analysis

Data obtained were analyzed statistically for descriptive statistics (mean, maximum, minimum and standard error) using SPSS 14 ***(23)***.

RESULTS

Total mould count/g of the examined samples

As revealed in Table (1) it was noticed that the highest total mould count/g was obtained from the sausage samples, followed by hawawchi samples, luncheon and minced meat; whereas the frozen meat samples yielded the lowest count of mould.

Table 1.Total mould count in meat and meat product samples

Type of examined sample	Total moulds count (TMC)			
	Min	Max	Mean	± SE
Frozen meat	$1X\ 10^2$	$2.60\ X\ 10^3$	$2\ X\ 10^2$	$1.20\ X\ 10^2$
Minced meat	$1X\ 10^2$	$1.50\ X\ 10^4$	$1.60\ X\ 10^3$	$6.00X\ 10^2$
Liver	$2X\ 10^2$	$4.60\ X\ 10^3$	$4.5\ X\ 10^3$	$9.50\ X\ 10^3$
Kidney	$1.20\ X\ 10^2$	$3.00\ X\ 10^3$	$2.20\ X\ 10^3$	$2.97\ X\ 10^3$
Luncheon	$1X\ 10^2$	$4.50\ X\ 10^4$	$3.50\ X\ 10^3$	$3.30\ X\ 10^3$
Sausage	$2X\ 10^2$	$5.40\ X\ 10^5$	$4.20\ X\ 10^4$	$1.25\ X\ 10^4$
Hawawchi	$3.50\ X\ 10^2$	$3.60\ X\ 10^5$	$5.20\ X\ 10^4$	$4.21x\ 10^4$

Min = minimum . Max = maximum. SE = standard error

Prevalence of moulds in meat and meat product samples.

From Table (2) and Fig (1), it is evident that nine genera were isolated and the most frequently encountered mould genera from the examined samples were *Aspergillus* and *penicillium*, while *Alternaria, Cladosporium, Fusarium, Mucor, Rhizopus, Scopulariopsis* and *Curvularia* species were recovered at low percentages.

Table 2. Prevalence of moulds in meat and meat product samples

Type of sample	Frozen meat		Minced meat		Liver		Kidney		Luncheon		Hawawchi		Sausage	
Identified mould spp.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
	50		50		50		50		50		50		50	
Aspergillus spp.	35/50	70	50/50	100	37/50	74	25	50	39	78	40	80	40	80
Penicillium spp.	25	50	10	20	8	16	20	40	19	38	17	34	25	50
Alternaria spp.	2	4	1	2	0	0	4	2	4	8	2	4	15	30
Cladosporium spp.	1	2	3	6	4	8	3	6	7	14	0	0	10	20
Curvularia spp.	1	2	1	2	0	0	0	0	1	2	0	0	2	4
Fusarium spp.	0	0	2	4	1	2	1	2	2	4	2	4	3	6
Mucor spp.	1	2	2	4	2	4	5	10	3	6	6	12	5	10
Rhizopus spp.	1	2	2	4	1	2	3	6	4	8	2	4	2	6
Scopulariopsis spp.	1	2	1	2	1	2	2	4	0	0	0	0	6	12

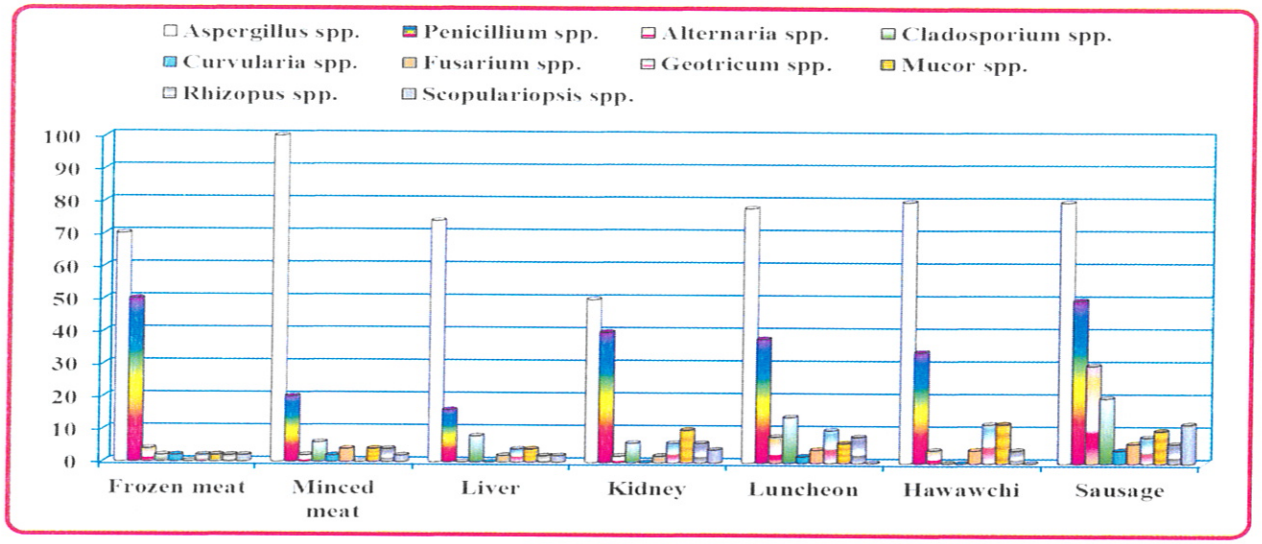

Fig. 1. Prevalence of mould in meat and meat product samples

Frequency of identified *Aspergillus* species isolated from examined meat and meat products

Aspergillus species were further identified. The most prevalent identified species were *A. flavus*, *A. niger* and *A. candidus* followed by *A. versicolor*, *A. fumigatus* , *A. ochraceus* , *A. parasiticus* and *A. terreus* as shown in table (3) and Fig.(2).

Screening of aflatoxin production on coconut agar medium:

For determination of aflatoxin production based on presence or absence of fluorescence on the reverse side of the colonies was determined by exposing the petri dishes to U.V. (365 nm) and expressed as positive (blue fluorescence) or negative as revealed in Fig. (3 & 4).

Table 3. Frequency of identified *Aspergillus* spp. isolated from the examined meat and meat products

Type of sample	Frozen meat		Minced meat		Liver		Kidney		Luncheon		Hawawchi		Sausage	
Aspergillus spp.	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
A. candidus	50		50		50		50		50		50		50	
	12	24	10	20	11	22	9	18	7	14	3	6	13	26
A. flavus	15	30	17	34	16	32	11	22	15	30	12	24	17	34
A. parasiticus	1	2	2	4	3	6	5	10	1	2	1	2	1	2
A. fumigatus	2	4	1	2	4	8	1	2	1	2	3	6	3	6
A. niger	12	24	15	30	15	30	13	26	12	24	14	28	5	10
A. ochraceus	1	2	10	20	6	12	8	16	4	8	3	6	3	6
A. terreus	0	0	1	2	1	2	0	0	1	2	1	2	1	2
A. versicolor	4	8	0	0	0	0	1	2	0	0	3	6	0	0

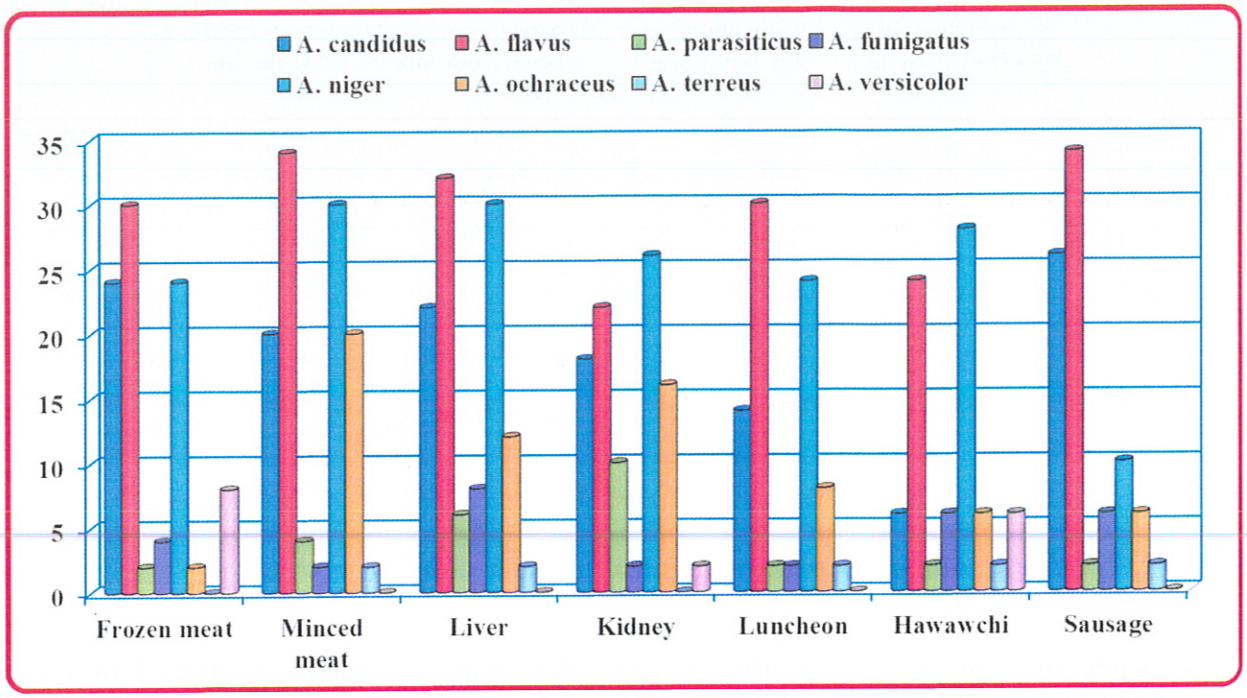

Fig. 2. Frequency of identified *Aspergillus* spp. isolated from the examined meat and meat products

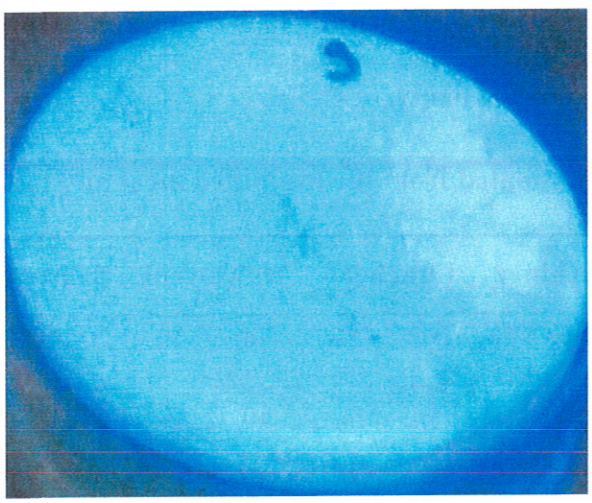

Fig.3. Aflatoxin producing *A. flavus* on CAM showing blue fluorescence on reverse side of the plate after exposure to u.v. rays

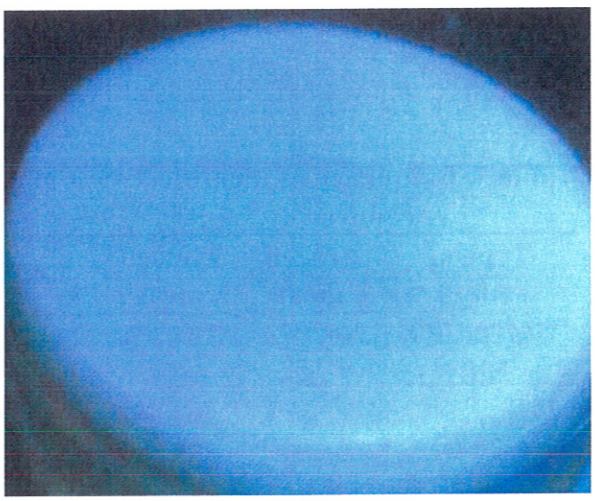

Fig.4. Negative control CAM plate

Aflatoxins detection from *A. flavus* and *A. parasiticus* strains (ug/ 1000 ml) (ppb)

Data presented in table (4) and Fig. (5) illustrate type (B_1, B_2, G_1 and G_2) and level of aflatoxins produced by *A. flavus* and *A. parasiticus* strains (ug/ 1000 ml)(ppb).

Aflatoxin residues (ppb) detected in examined meat and meat product samples

As demonstrated in table (5) and fig (6), the highest mean values of aflatoxin residues (µg/kg), B1, B2, G1 and G2 were detected in the kidney samples (12.36 ± 1.89, 9.84 ± 1.63, 5.38 ± 1.36 and 6.84 ± 1.39, respectively) and the lowest level was detected in frozen meat samples (4.80 ± 0.89, 5.3 ± 2.1, 1.71 ± 0.60 and 0.0) respectively.

Table 4. Aflatoxins detection from strains of *A. flavus* and *A. parasiticus* isolated from meat and meat product samples (µg/ 1000 ml) (ppb)

Source of isolation	Strains	Total No. of isolates	Aflatoxin +ve isolates		Quantitative detection of aflatoxins (µg/1000 ml) (ppm)			
			No	%	B_1	B_2	G_1	G_2
Frozen meat	*A. flavus*	8	7	87.5%	3.5-66.0	10.5-37	0	0
	A. parasiticus	1	1	100%	43.0	32	0-43	0-26
	Total	9	8	88.8				
Minced meat	*A. flavus*	10	8	80%	0-31	0-22	0	0
	A. parasiticus	0	0	0	0	0	0-33	0-16
	Total	10	8	80%				
Liver	*A. flavus*	9	7	77.7%	0-37	0-36	0	0
	A. parasiticus	1	1	100.0%	55	22	0-77	0-35
	Total	10	8	80%				
Kidney	*A. flavus*	6	5	83.3%	0-15	0-20	0	0
	A. parasiticus	1	1	100.00%	12	0	18	16
	Total	7	6	85.7%				
Luncheon	*A. flavus*	8	5	62.5%	12-24	0-20	0	0
	A. parasiticus	0	0	0	0	0	0-27	0-27
	Total	8	5	62.5%				
Sausage	*A. flavus*	11	9	81.8%	9.5-25	0-23	0	0
	A. parasiticus	0	0	0	0	0	0	0-9
	Total	11	9	81.8%				
Hawawchi	*A. flavus*	7	5	71.4%	0-41	0-26	0	0
	A. parasiticus	0	0	0	0	0	0	0
	Total	7	5	71.4%				

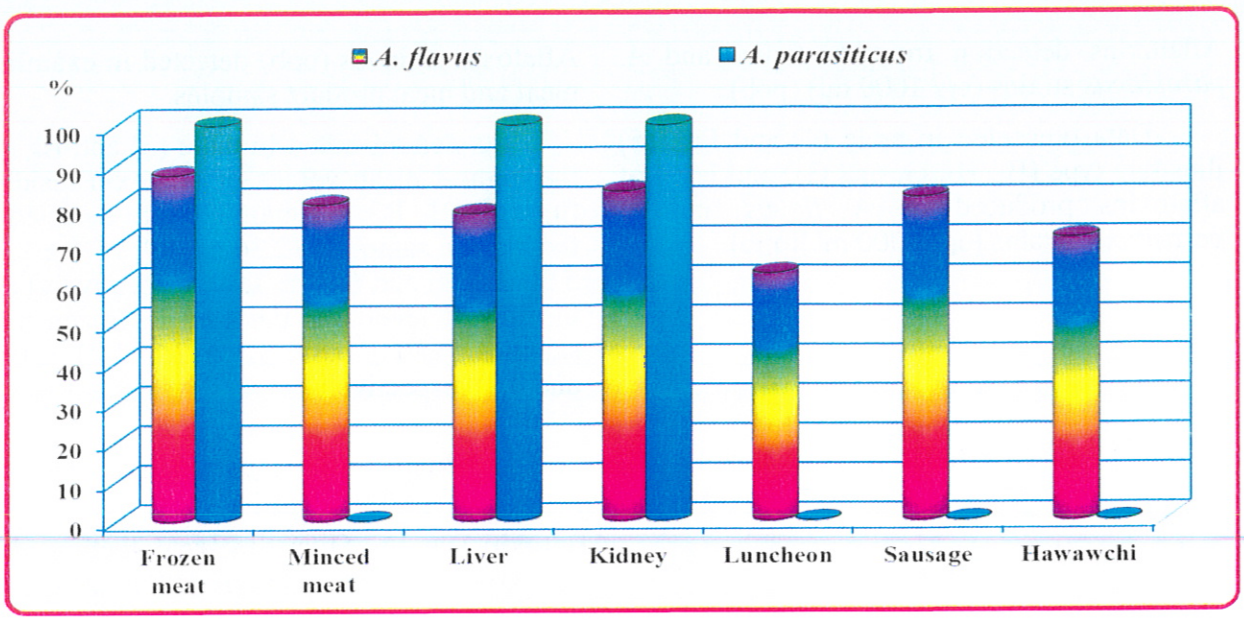

Fig. 5. Prevalence and levels of aflatoxins produced by *A. flavus* and *A. parasiticus* that isolated from examined meat and meat product samples (ppm).

Comparison between the total aflatoxins residues detected in examined samples of meat and meat products and the permissible limits of WHO (15 ppb) and FAO & FDA (20 ppb).

The detected levels of aflatoxin residues in the present samples were compared with the international permissible limits of WHO (1975), FAO (1995) and FDA (1999). Most of detected aflatoxins levels in meat and meat product samples were more than the permissible limits thus would be unfit for human consumption as revealed in table (6).

Table 5. Statistical analysis of aflatoxin residues (ppb) detected in examined meat and meat product samples (n=20)

Types of aflatoxins	B_1				B_2				G_1				G_2			
Type of examined sample	Min	Max	Mean	±SE	Min	Max	Mean	±SE	Min	Max	Mean	±SE	Min	Max	Mean	±SE
Frozen meat	1.2	9.3	4.8	0.9	0	22	5.3	2.1	0	6	1.71	0.6	0	0	0	0
Minced meat	0	7.5	3.62	0.9	0	7.7	3.4	0.8	0	8.1	4.24	0.9	0	5.5	2.83	0.6
Liver	0	20	13.8	2	0	6.3	3.26	0.6	0	7	2.51	0.6	0	3.5	1.36	0.4
Kidney	3.8	24	12.4	1.9	3.8	18	9.84	1.6	1.5	18	5.38	1.4	1.5	16	6.84	1.4
Luncheon	0	11	3.71	1.4	0	11	3.59	1.1	0	11	5.24	1.1	0	15	6.77	1.5
Sausage	0	19	6.05	2.1	0	8.5	2.2	0.8	0	15	5.34	1.5	0	6.8	2.36	0.7
Hawawchi	0	21	11	2.4	0	5	2.25	0.5	0	11	2.54	1	0	8.5	2.56	0.8

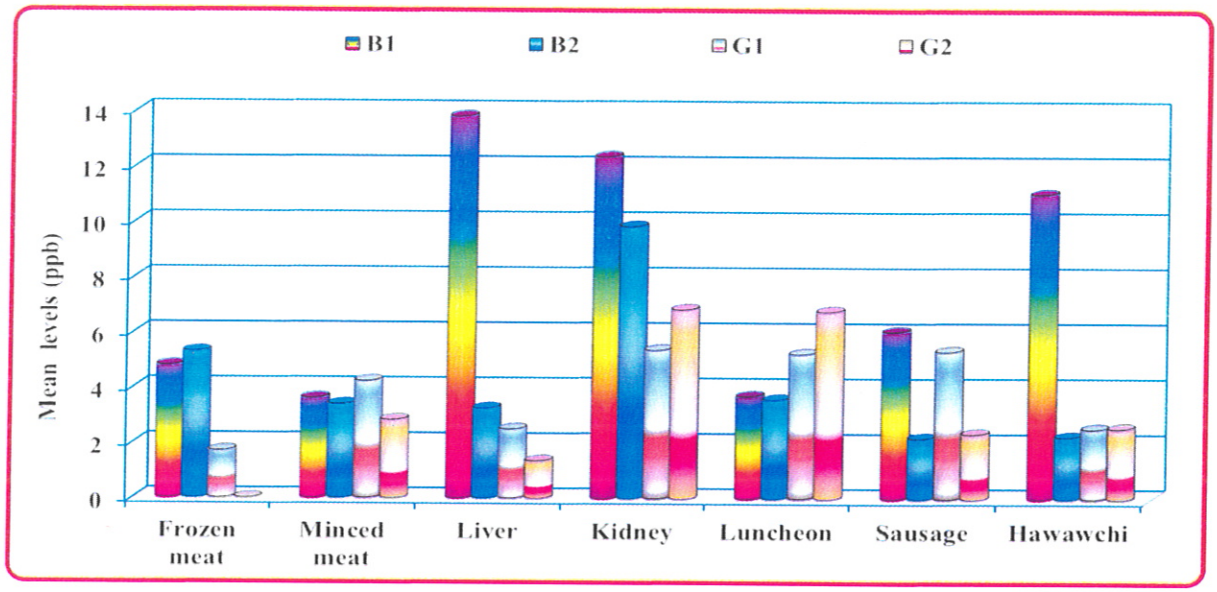

Fig. 6. Statistical analytical results of various aflatoxin residues (ppb) detected examined meat and meat product samples (n=15)

Table 6. Comparison between the total aflatoxin residues detected in meat and meat products and recommended permissible limits of WHO *(39)*, FAO *(40)* and FDA *(41)*.

Type of examined sample	No. of examined samples	No. of positive samples	Samples contained aflatoxins> 15 ppb (WHO)		Samples contained aflatoxins > 20 ppb (FAO) & FDA	
			No.	%	No.	%
Frozen meat	20	11	5	25	2	10
Minced meat	20	15	7	35	4	20
Liver	20	11	8	40	5	50
Kidney	20	13	9	45	7	35
Luncheon	20	15	13	65	8	40
Sausage	20	17	15	75	6	30
Hawawchi	20	14	7	35	6	30

DISCUSSION

The consumption of food contaminated with moulds and their toxic metabolites results in the development of food-borne mycotoxicosis ***(4)***. Advanced countries considered the mould counts as a standard test for hygienic condition due its economic and public health effects ***(24)***.

In the present study, the highest total mould count/g was obtained from the sausage samples (ranged from 2 X 10^2 to 5.40 X 10^5 with a mean count of4.20 X 10^4 ± 1.25 X $10^{4)}$, followed by hawawchi, luncheon and minced meat samples; whereas the frozen meat samples yielded the lowest mould count ranged from 1 X 10 to 2.60 X 10^3 with a mean

count of 2.0 X 10^2 ± 1.2 X 10^2. These results come in accordance with those reported by *(25-28)*. The high incidence of moulds in sausage and other meat products may be due to the frequent unhygienic handling and processing of meat especially when additives of low quality as flavorings, especially, spices were used *(4,29)*.

The most common species were *Aspergillus* which isolated from hawachi and sausage samples at percent of 80%, followed by liver (74%), frozen meat (70%) and kidney samples (50%).Among *Aspergillus* species, *A. flavus* was the most predominant in minced meat and sausage samples (34% of each), liver, (32%), frozen meat and luncheon samples (30% of each), in hawawchi samples it was (24%), and recovered from kidney samples at the rate of (22%).

It is interested to denote that *A. parasiticus* was isolated in low incidence in comparison with that of *A. flavus.* Where, its incidence rate in kidney was (10%), in liver was (6%) and in minced meat samples was (4%). While in luncheon, frozen meat, hawawchi and the sausage the rates of isolation were (2% for each). Under the same conditions, other members of *Aspergillus* were recovered in variable frequencies. Nearly similar results were obtained by ***(4, 13,28,30, 31, 32)***.

Aspergillus was known to common contaminants of human foods and animal feeds (*33*). The presence of Aspergillus is not only of economic important but also represents a real health hazard. They can be of allergic, toxigenic and pathogenic effect through the production of mycotoxins ***(34)***.

Aflatoxin production was screened on coconut agar medium (CAM) on the basis of the presence of aflatoxin in blue-fluorescing agar plates and its absence in non-fluorescing one as confirmed by TLC and fluoroumetric analysis. This result is in consistent with those obtained by ***(35, 36)***.

The TLC and fluoroumetric detection of aflatoxins (Bl, B2, Gl and G2 (ppb)produced by *A. flavus* and *A. parasiticus* isolates and aflatoxin residues (µg/kg) in meat and meat product samples as shown in table (4&5) revealed that *A. flavus* (87.5%) and *A. parasiticus* (100%) that recovered from frozen meat produced significant levels of aflatoxins ranged from (3.5 -66.0 ppb) for AFB1, and (10.5-37.0 ppb) for AFB2 by *A. flavus*, while AFs by *A. parasiticus* ranged from (43.0 ppb) for AFB1,(32.0 ppb) for AFB2 , (0 - 43.0 ppb)for AFG1 and (0 - 26.0 ppb) for AFG2. These results were similar to those reported by ***(32)*** who isolated aflatoxigenic *Aspergillus* species from imported frozen meat while (***31***)found the highest average quantity of aflatoxins was in sausage and beef burgers samples.

The highest mean values of aflatoxin residues (µg/kg) B_1, B_2, G_1 and G_2 were detected in the kidney samples (12.36 ± 1.89, 9.84 ± 1.63, 5.38 ± 1.36 and 6.84 ± 1.39) respectively and frozen meat samples had the lowest level of aflatoxin (4.80 ± 0.89, 5.3 ± 2.1, 1.71 ± 0.60 and 0.0) respectively. These findings agreed with hat recorded by ***(13);*** while ***(26)*** recorded extreme higher incidence attained to 100%, and ***(37)*** recorded a relatively higher incidence, but lower than obtained by ***(38,26)***.

Food and Drug Administration (FDA) established regulatory working guidelines on the acceptable levels of aflatoxins in human foods set at 20 ppb for total aflatoxins, with the exception of milk which has an action level of 0.5 ppb of aflatoxins (*33*). The detected levels of aflatoxin residues in the present samples were compared with the international permissible limits of WHO ***(39)***, FAO ***(40)*** and FDA ***(41)***. In sausage samples showed residues of aflatoxins in 75% of samples more than the permissible limits of 15 ppb (WHO) and 30% of samples contained aflatoxins more than the permissible limits of 20 ppb (FAO).In luncheon samples, aflatoxins residues were in 65% of samples more than the permissible limits of 15 ppb (WHO) and 40% of samples contained aflatoxins more than the permissible limits of 20 ppb (FAO) thus would be unfit for human consumption.

Conclusion

The most effective mean to prevent aflatoxigenic mould contamination of meat products is through application of strict hygienic measures during the processing of meat products and using a good quality flavoring agents as spices.

REFERENCES

1. ***Bhat R, Rai R V and Karim A A (2010):*** Mycotoxins in Food and Feed: Present Status and Future Concerns. Comprehensive reviews in food science and food safety, 9: 57-81.

2. ***Wogan GN, Paglialunga S, Newberue PN. (1974):*** Carcinogenic effect of low dietary levels of aflatoxin B1 in rats. Food Cosmet Toxicol.,12: 681–685.

3. ***Pitt JI, Hocking AD. (1997):*** Fungi and food spoilage. 2nd ed. Blackie Academic & Professional, London, U.K., 593 pp.

4. ***Mizakova A, Pipova Mand Turek P (2002):*** The occurrence of moulds in fermented raw meat products. Czech J. Food Sci., 20: 89–94.

5. ***Jesenska Z (1987):*** Mikroskopickéhuby v požívatinách a v krmivách. Alfa, Bratislava: 36–37.

6. ***Štyriak I, Čonkova E, Laciakova A, Bohm J (1998):*** Prevention of fumonisin production by microorganisms.Czech J. Anim. Sci., 43: 449–452.

7. ***Hanssen H P (1995):*** Mould control in the meat processing industry. Fleischwirtschaft, 75: 52.

8. ***Frisvad JC and Samson R A (1991):*** Filamentous fungi in foods and feeds: ecology, spoilage, and mycotoxin production. In D. K. Arora, K. G. Mukerji, & E. H. Marth (Eds.), Handbook of applied mycology: Foods and feeds (pp. 31e38). New York:Marcel Dekker, Inc.

9. ***Bailly J D, Tabuc C, Quérin A, and Guerre P (2005):*** Production and stability of patulin, ochratoxin A, citrinin, and cyclopiazonic acid on dry cured ham. Journal of Food Protection, 68, 1516-1520.

10. ***Núñez F, Díaz M C, Rodríguez M, Aranda E, Martín A andAsensio M A (2000):*** Effects of substrate, water activity, and temperature on growth and verrucosidinproduction by Penicillium polonicum isolated from dry-cured ham. Journal of Food Protection, 63, 232-236.

11. ***Núñez F, Westphal, C D, Bermúdez E andAsensio M A (2007):*** Production of secondary metabolites by some terverticillate Penicillia on carbohydrate-rich and meat substrates. Journal of Food Protection, 70: 2829-2836.

12. ***Mateo E, Gil-Serna J, Patiño B, & Jiménez M (2011):*** Aflatoxins and ochratoxin A in stored barley grain in Spain and impact of PCR-based strategies to assess the occurrence of aflatoxigenic and ochratoxigenic *Aspergillus* spp. International Journal of Food Microbiology, 149:118-26.

13. ***Aziz N H and Youssef AY (1991):*** Occurrence of aflatoxins and aflatoxin-producing moulds in fresh and processed meat in Egypt. Food Additives & Contaminants, 8: 321-331.

14. ***Trucksess MW, Richard JL, Stoloff L, McDonald JS and Brumley WC. (1988):*** Effect of the temperature, water activity and the growth of *A. flavus* and aflatoxin production on corn, pinto beans and soybeans. J. Food Protect., 51: 361-363.

15. ***International Commition on Microbiological Specifications for foods (ICMSF) (1978):*** Microbiology in foods, their significance and methods of enumeration, 2nd Ed. University of Toronto press, Canada.

16. ***Raper KBand FennelDI (1965):*** The genus Aspergillus. Pp.686. Baltimore, Williams and Wilkins.

17.Pitt JI and Hocking AD (2009): Fungi and food Spoilage Book . 4rd Ed., Chapter 4-10, pp. 29. Academic Press, New York, London, Toronto, Montreal, Tokyo.

18.Davis ND, Iyer SK and Diener UL (1987): Improved method of screening for a¯atoxin with a coconut agar medium. Applied and Environmental Microbiology 53, 1593-1595.

19.Bauer J, Montgelas AV and Gedleck B (1983): Aflatoxin B production in presence of preservatives antimicrobial agent. Pro. Int. Symp. Mycotoxins, Cairo, Egypt, pp. 249-255.

20.Gabal MA, Hegazi SM and Hassanien Y (1994): Aflatoxin production by *Aspergillus flavus* field isolates. Vet. Human Toxicol., 36: 519- 521.

21.Roberts B A and Patterson D S P (1975): Detection of twelve mycotoxins in mixed animal Feed stuffs using novel membranes clean up Procedure. J. Assoc. Off. Anal. Chem., 58: 1178.

22.Association of Official Analytical Chemists (AOAC) (1995): Official methods of analysis (Association of Official Analytical Chemist), 16th Ed. chapter 10, International, Gaithersburg. Inc. Suite 400, 2200 Wilson Boulevard Arlington, Virginia 22201, USA.

23.SPSS 14 (2006): Statistical Package for Social Science, SPSS for windows Release 14.0.0, 12 June, 2006. Standard Version, Copyright SPSS Inc., 1989-2006, All Rights Reserved, Copyright ® SPSS Inc.

24.Ismail MA, AbouElala AH, NassarA and Michail DG (1995): Fungal contamination of beef carcasses and the environment in a slaughter house. Food Microbiology, 12: 441- 445.

25.Mansour NK, Yassien NA and El-Sherif AM (1994): Mycoflora of imported frozen meat. Vet. Med. J. Giza, Egypt., 42: 94-98.

26.El- Gazzar, M.M. (1995): Mycological studies on some meat products. Ph. D. Thesis, Fac. Vet. Med., Zagazig University Egypt.

27.Nouman, T, Darwish, A, Zinab, M. Niazi; and Hoda, A. Aiedia (2001): The traditional Egyptian luncheon 1- Quality attributes of market product. Vet. Med. J. Giza, 49: 199- 210.

28.El- Tabiy AA (2006): Mycological study on some processed meat products exposed for sale in markets. Assiut Vet. Med. J., 52:121-131.

29.Wafia HA and Hassan AA (2000): Sanitary status of some to eat meat meals in Cairo and Giza Governorates. J. Egypt. Vet. Med. Assoc., 60: 95- 104.

30.Ismail M A and Zaky ZM (1999): Evaluation of the mycological status of luncheon meat with special reference to aflatoxigenic moulds and aflatoxin residues. Mycopathologia, 146: 147- 154.

31.Brr AH, MoustafaNY and EdrisAM (2004): Incidence of Moulds and Aflatoxiins in Some Meat Products, Benha Vet. Med.J., 15:65-75.

32.Hassan A MS (2013): Frequency rates of fungal contaminants in imported meats from alexandrian retail markets. Life Science Journal,10:158-165.

33.Bullerman LB (1979): Significance of mycotoxins to food safety and human health. J. Food Protect. 24:65-86.

34.Aldoory Y (1980): Studies on the proteolytic activity of some prevalent mould species. Lab. Med. Mycology, Lea & Febryev. Philadelphia.

35.Rodrigues P, Soares C, Kozakiewicz K, Paterson R R, Lima N and Venâncio A (2009): Identification and characterization of *Aspergillus flavus* and aflatoxins. Applied Microbiology, 2: 527-534 .

36.Jamali M, Karimipour M, Shams M, Amani A and Abyaneh M (2013): Expression of aflatoxin genes aflO (omtB) and aflQ (ordA) differentiates levels of aflatoxin production by *Aspergillus flavus*

strains from soils of pistachio orchards. *Research in Microbiology*,164: 293-299.

37. ***Roushdy S, Ibrahim A, Al-Danaf N, Hammad H and Moustafa R (1996):*** Mycotoxin residues in meat products. Vet. Med. J. Giza, 44: 181-187.

38. ***El- Shawaf A M (1990):*** Microbial studies on aflatoxins in meat products. Ph. D. Thesis (Food Technology), Fac. of Agriculture, Mansoura Univ., Egypt.

39. ***WHO (World Health Organization) (1979):*** Environmental health criteria II, Mycotoxins, Geneva, 86-98.

40. ***FAO (Food and Agriculture Organization of the United Nations) (1995):*** Food and Nutrition paper. Worldwide Regulation of Mycotoxins, Advanced Copy.

41. ***Food and Drug Administration F.D.A (1999):*** Action levels for poisonous or deleterious substances in human food and animal feed. Download from: http:/Vm.Cfsan.fda gov/ ird/ fdaact.html on 7/8/1999.

الملخص العربى

الأعفان المفرزة لسموم الافلاتوكسين و بقاياها فى عينات اللحوم و منتجاتها فى مصر

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** قسم الفطريات و السموم الفطريه- معهد بحوث صحة الحيوان - دقى- جيزة

استهدفت هذه الدراسة القاء الضوء على معدلات تلوث اللحوم ومنتجاتها بالأعفان المفرزة لسموم الافلاتوكسين و بقاياها بعينات اللحوم و منتجاتها. وقد تم اجراء هذه الدراسة على ٣٥٠ عينة من اللحوم ومنتجاتها تشمل ٥٠ عينة من كل من اللحوم المجمدة و اللحوم المفرومة و الكبدة و الكلية و اللانشون و السجق و الحواوشي.

تبين أن أعلى نسبة تلوث بالأعفان فى عينات اللحوم تتواجد فى عينات السجق المفروم وكان اللحم المجمد اقلهم تلوثا. وبتصنيف اجناس الاعفان الموجودة باللحوم ومنتجاتها تبين ان أعلى عزل لأجناس الأعفان هو عفن الاسبرجيلس و البنسيليم.تم عزل فصائل الاسبرجلس بنسبة ١٠٠% ، ٨٠% ، ٨٠% ، ٧٨% ، ٧٤% ، ٧٠% و٥٠% من كل من اللحوم المفرومة و الحواوشي و السجق و اللانشون و الكبدة و اللحوم المجمدة و الكلية بالترتيب. وكان الاسبرجيلس فلافس اكثر العترات التى تم التعرف عليها.كل عترات الاسبرجيلس براسيتكس و ٧٧,٩٦% من عزلات الاسبرجيلس فلافس قادره على افراز سموم الافلاتوكسين التى تم الكشف عنها باستخدام وسط جوز الهند (CAM)، (TLC) وطريقة (fluorometric immunoaffinity). تم الكشف عن الافلاتوكسين B1، B2، G1 و G2 وبقاياها بعينات اللحوم و منتجاتها و تبين أن أعلى متوسط لقيم بقايا الأفلاتوكسين (ميكروغرام /كغ) في عينات الكلى(١,٨٩ ± ١٢,٣٦، ١,٦٣ ± ٩,٨٤، ١,٣٦ ± ٥,٨٣ و ١,٣٩±٦,٨٤) على التوالي، وعينات اللحوم المجمدة لديها أدنى مستوى من الافلاتوكسين ٠,٨٩ ±٤,٨٠، ٢,١ ± ٥,٣، ٠,٦٠± ١,٧ و ٠,٠)على التوالي.تم عمل مقارنة بين بقايا سموم الافلاتوكسين فى العينات والحد المسموح به دوليا طبقا لمنظمة الصحة العالمية(١٥ جزء في البليون) ،الفاو وادارة الاغذية والعقاقير(٢٠جزء في البليون) ووجد انها أعلى من الحدود المسموح بها وبالتالي تكون غير صالحة للاستهلاك البشري. و بالتالى هذه الدراسه تجذب الانتباه الى المخاطر المحتملة للأعفان المفرزة لسموم الافلاتوكسين والأفلاتوكسين التى تلوث اللحوم و منتجاتها وتوصي بقوة تجنب الاسباب المختلفة للتلوث مثل عدم سلامة الاجراءات الصحية أثناء الذبح، والنقل والتخزين أو المعالجة للحوم ومنتجاتها وأيضا تجنب التوابل الملوثة المستخدمة في تجهيز اللحوم.